PATENT
Docket No. 511582001620

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Ruth Sysko

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Daniel E. H. AFAR, *et al.*

Serial No.: 09/455,486

Filing Date: 6 December 1999

For: NOVEL SERPENTINE TRANSMEMBRANE ANTIGENS EXPRESSED IN HUMAN CANCERS AND USES THEREOF

Examiner: Gary B. Nickol, Ph. D.

Group Art Unit: 1642

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DECLARATION OF MARY FARIS

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Mary Faris, declare as follows:

1. I hold the position of Group Leader-Target Validation at Agensys, Inc. This position requires me to generate *in vitro* models for the study of cancer, and to investigate the effect of specific genes and gene products on tumor development, growth, and progression. This position also requires me to attend national and international conferences addressing issues in cancer research, and exhibiting established as well as cutting edge ideas. I have a Ph.D. in Immunology and Microbiology from Ohio State University, and have held two postdoctoral fellowships, one at the University of Virginia and one at the University of California at

Los Angeles, School of Medicine and have worked in the field of molecular biology for over 10 years. A copy of my *curriculum vitae* is attached as Exhibit A.

2. In connection with my work at Agensys, I performed certain experiments regarding the expression of STEAP-2 protein which is identified in the above-referenced application as having the amino acid sequence shown in Figure 9 and encoded by a cDNA insert in a plasmid deposited with the American Type Culture Collection as ATCC Accession No. PTA-311. As deduced from the coding sequence, the open reading frame encodes 454 amino acids with 6 transmembrane domains. A summary of the characteristics associated with STEAP-2 protein is shown on page 1 of Exhibit B.

3. The data set forth in the present patent application provide an expression profile of the STEAP-2 protein that is predominantly specific for the prostate among normal tissues, for certain types of prostate tumors as well as other tumors. This evidence is based on detecting messenger RNA using Northern blotting. We, in keeping with standard practice in our industry, use Northern blots routinely to assess gene expression, as it does not require the time consuming process of synthesizing the relevant protein, raising antibodies, assuring the specificity of the antibodies, required for Western blotting of proteins and the histological examination of tissues. Northern blotting offers a credible and efficient method of assessing RNA expression and expression levels.

4. I understand that the Patent Office has questioned whether this evidence is probative of the production of the STEAP-2 protein itself. The experiments that I will describe in this Declaration demonstrate that STEAP-2 protein is, indeed, produced. In summary, the experiments show that PC-3 cells and 3T3 cells which were modified to contain an expression system for STEAP-2 showed enhanced levels of tyrosine phosphorylation in general, and of phosphorylation of ERK protein in particular. The data also show that PC-3 cells that contain an expression system for STEAP-2 showed modified calcium flux, a modified response to paclitaxel, and a general inhibition of drug-induced apoptosis. These are effects exhibited at the protein level, thus these data alone are, in my opinion, probative that the STEAP-2 protein exists. Furthermore, although such phenotypic effects are protein-

mediated, further evidence indicates that the STEAP-2 protein itself is the mediator of the effects. This evidence is obtained by utilizing a modified STEAP-2 protein. An expression system is stably introduced into PC3 and 3T3 cells which allows the expression of a modified form of STEAP-2, designated STEAP-2CFl. STEAP-2CFl is a STEAP-2 protein having a peptide extension, i.e., a Flag epitope that alters the physical conformation of this protein. The Flag epitope is a string 8 amino acids, often introdueed at either the amino or carboxy termini of protein as a means of identifying and following a recombinant protein in engincered cells (Slootstra JW et al, Mol Divers 1997, 2:156). In most cases, the introduction of the Flag epitope at either termini of a protein has little effect on the natural function and location of that protein (Molloy SS et al, EMBO J 1994, 13:18). However, this is dependent on the characteristics of the protein being Flag tagged. Recent studies have shown that a Flag tag affects the function and conformation of select proteins such as the CLN3 protein (see, e.g., Haskell RE, et al. Mol Genet Metab 1999, 66:253). As with CLN3, introducing a Flag epitope tag to the C-terminus of STEAP-2 alters the physical conformation and properties of this protein. Altering the STEAP-2 protein with the C-Flag epitope resulted in a significant decrease in the effects otherwise observed, including phosphorylation of ERK and resistance to drug-induced cell death. The data indicate that it is the STEAP-2 protein that mediated these phenotypic effects. Finally, *in vitro* translation studies using rabbit reticulocyte lysate, show that the STEAP-2 protein is translated and exhibits the expected molecular weight.

5. Turning, now to the experimental work itself, pages 2 and 3 of Exhibit B show the results obtained when PC-3 and 3T3 cells, respectively, were modified to contain the retroviral expression system pSRα encoding the indicated proteins, including STEAP-1, STEAP-2 and STEAP-2CFl, respectively. Gene-specific protein expression was driven from a long terminal repeat (LTR), and the Neomycin resistance gene was used for selection of mammalian cells that stably express the protein. PC-3 and 3T3 cells were transduced with the retrovirus, selected in the presence of G418 and cultured under conditions which permit expression of the STEAP-2 coding sequence. The cells were grown overnight in low concentrations of FBS (0.5-1% FBS) and were then stimulated with 10% FBS. The cells were lysed in RIPA buffer and quantitated for protein concentration. Whole cell lysates were

separated by SDS-PAGE and analyzed by Western blotting using anti-phospho-ERK (Cell Signaling Inc.) or anti-phosphotyrosine (UBI) antibodies (Exhibit B pages 2, 3 and 4). As shown on Exhibit B page 2, as compared to untransformed PC-3 cells, cells modified to contain STEAP-2 contain enhanced amounts of phosphorylated tyrosine. Similar results from an analogous experiment on 3T3 cells are shown on page 3. In this latter experiment, the STEAP-2CFl expression system was also transfected into 3T3 cells, which cells were used as a control. As shown on Exhibit B page 3, the enhanced phosphorylation found in the presence of native STEAP-2 was significantly reduced when the conformation of the protein was altered. These results thus show conclusively that the STEAP-2 protein was produced and mediated the above-described phenotypic effects.

6. Page 4 of Exhibit B shows similar results, both in PC-3 and 3T3 cells where phosphorylation of ERK, specifically, is detected. The protocol is similar to that set forth in paragraph 5 above, except that rather than probing the gels with antibodies specific for phosphotyrosine the gels were probed both the anti-ERK and anti-phospho-ERK antibodies. As shown on Exhibit B page 4, in the presence of 10% FBS, both PC-3 cells and 3T3 cells modified to express STEAP-2 showed phosphorylation of ERK which was not detectable in cells transformed to contain STEAP-2CFl. In contrast to control PC-3 cells which exhibit no background ERK phosphorylation, control 3T3-neo cells show low levels of endogenous ERK phosphorylation. Treatment with 10% FBS enhanced phosphorylation of ERK protein in cells expressing STEAP-2 relative to 3T3-neo cells, while no increase in ERK phosphorylation was observed in 3T3 cells expressing modified STEAP-2, i.e. STEAP-2 CFl.

7. Other effects on cellular metabolism in cells modified to contain a STEAP-2 expression system were also shown in our data. Page 5 of Exhibit B shows that when cells with and without expression systems for STEAP-2 were measured for calcium flux in the presence of LPA, calcium flux was enhanced in the STEAP-2 containing cells. Using FACS analysis and commercially available indicators (Molecular Probes), parental cells and cells expressing STEAP-2 were compared for their ability to transport calcium. PC3-neo and PC3-STEAP-2 cells were loaded with calcium responsive indicators Fluo4 and Fura red, incubated in the

presence or absence of calcium and LPA, and analyzed by flow cytometry. PC3 cells expressing a known calcium transporter, PC3-83P3H3 pCaT were used as positive control (Biochem Biophys Res Commun. 2001, 282:729). The table on Exhibit B page 5 shows that STEAP-2 mediates calcium flux in response to LPA, and that the magnitude of calcium flux is comparable to that produced by a known calcium channel. In addition, STEAP-2 expressing PC3 cells demonstrated increased sensitivity to agatoxin, a calcium channel blocker as compared to PC3-neo cells. These results indicate that STEAP-2 expression renders PC3 cells sensitive to treatment with the Ca++ channel inhibitors. Information derived from the above experiments provides a mechanism by which cancer cells are regulated. This is particularly relevant in the case of calcium, as calcium channel inhibitors have been reported to induce the death of certain cancer cells, including prostate cancer cell lines (see, e.g., Batra S, Popper LD, Hartley-Asp B. Prostate. 1991, 19:299).

8. Page 6 of Exhibit B shows that cells transfected with a STEAP-2 expression system have enhanced ability to survive exposure to paclitaxel. In order to determine the effect of STEAP-2 on survival, PC3 cells lacking or expressing STEAP-2 were treated with chemotherapeutic agents currently used in the clinic. Effect of treatment was evaluated by measuring cell proliferation using the Alamare blue assay (Example B page 6). While only 5.2% of PC3-neo cells were able to metabolize Alalmare Blue and proliferate in the presence of 5 μM paclitaxel, 44.8% of PC3-STEAP-2 cells survived under the same conditions. These results indicate that expression of STEAP-2 imparts resistance to paclitaxel. These findings have significant *in vivo* implications, as they indicate that STEAP-2 provides a growth advantage for prostate tumor cells in patients treated with common therapeutic agents.

9. A more detailed form of these results is shown on pages 7 and 8 of Exhibit B. Results in these two pages demonstrate the mode of action by which STEAP-2 supports the survival of PC3 cells. In these studies, PC3 cells expressing or lacking STEAP-2 were treated with paclitaxel for 60 hours, and assayed for apoptosis using annexin V conjugated to FITC and propidium iodide staining. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the membrane, thereby exposing PS to the external cellular environment. PS is recognized by and binds to annexin V, providing

scientists with a reliable means of identifying cells undergoing programmed cell death. Staining with propidium iodide identifies dead cells. The figures on page 7 show that expression of STEAP-2 inhibits paclitaxel-mediated apoptosis by 45% relative to paclitaxel-treated PC3-neo cells. The protective effect of STEAP-2 is inhibited when STEAP-2 is modified by the presence of Flag at its C-terminus (Exhibit B page 8).

10. The publicly available literature contains several examples of prostate and other cancers that exhibit similar phenotypic characteristics as those observed in PC3 cells that express STEAP-2. In particular, clinical studies have reported transient tumor regression and/or only partial responses in patients treated with paclitaxel. For instance, only around 50% of prostate cancer patients entered in a single agent clinical trial of paclitaxel showed reduced PSA levels when treated with doses of paclitaxel that induced grade 3 and grade 4 toxicity; a much higher level of response would have been expected based on this dose level, thus this data indicates the development of paclitaxel resistance in prostate cancer patients (Beer TM et al, Ann Oncol 2001, 12:1273). A similar phenomenon of reduced responsiveness and progressive tumor recurrence was observed in other studies (see, *e.g.*, Obasaju C, and Hudes GR. Hematol Oncol Clin North Am 2001,15:525). In addition, inhibition of calcium flux in cells that endogenously express STEAP-2, such as LNCaP cells, induces their cell death (Skryma R et al, J Physiol. 2000, 527:71).

11. I conclude from these results that STEAP-2 protein is produced not only in the cells tested, but also in unmodified tumor cells or unmodified prostate cells where the presence of mRNA has been shown. The Northern blot data in the specification clearly show that the messenger RNA encoding STEAP-2 is produced in certain prostate and tumor cells. The 3T3 and PC-3 cells, which are themselves tumor cell lines, are clearly able to translate the messenger RNA into protein. Because it has been shown that there is no barrier to translation of the message in cells similar to those tumor and prostate cells in which the mRNA has been shown to be produced, it can properly be concluded that the protein itself can be detected in the unmodified tumor or prostate cells, given the fact that it is shown that mRNA is produced. This conclusion is also supported by the patterns of phenotypic changes seen in cells

specifically modified to express STEAP-2, these changes comport with changes seen in cancer cells.

12. Based on the experiments above, therefore, I conclude that it is a sound conclusion scientifically that cells and tissues which produce mRNA encoding STEAP-2 also produce the protein itself.

13. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed at Santa Monica, California on 3 June 2002.

Mary Faris, Ph.D.

MARY FARIS, PH.D.

ADDRESS: 2538 Almaden Court
Los Angeles, CA 90077
310-859-8508

Agensys Inc.
1545 17th Street
Santa Monica, CA90404
310-820-8029

EDUCATION:

1986: B.S. with Distinction,
University of the State of New York

1991: Ph.D. in Immunology and Microbiology
The Ohio State University
Dissertation Title: Characterization of the Mechanism of Persistent I-A Expression by Macrophages.
Advisor: Dr. Bruce S. Zwilling

1991-1994: Postdoctoral Fellowship, University of Virginia.

1994-1996: Postdoctoral Fellowship, UCLA School of Medicine.

EXPERIENCE:

1987-1989: Teaching Assistant, The Ohio State University.
Microbiology in Relation to Man (Micro 509).
General Microbiology (Micro 520).
Principles of Infection and Immunity (Micro 522).
Cellular Aspects of the Immune System (Micro 632).

1989-1991: Graduate Research Associate, The Ohio State University.
Sponsor: Dr. B. Zwilling
Research Interests: Regulation of MHC class II Expression by IFNγ-mediated Pathways.

1991-1994: Postdoctoral Fellow, University of Virginia.
Sponsor: Dr. S. M. Fu.
Research Interests: CD40-Mediated Signal Transduction in lymphocytes.

1994-1996: Postdoctoral Fellow, UCLA School of Medicine.
Sponsor: Dr. A. Nel.
Research Interests: Signaling Pathways Mediating T cell Activation.
Signaling Pathways Involved in the Growth of Kaposi's Sarcoma tumors.

1996-1998: Researcher, Faculty, UCLA School of Medicine.
Research Interests: Mechanism of T cell Activation and Apoptosis.
Integration of multiple factors in the angiogenesis/tumorigenesis of Kaposi's sarcoma lesions.

Exh. 6, +D

1998-1999: Senior Scientist, Incyte Genomics.
Reserch Interests: Mechanism of Tumor Growth and Progression.
Integrated approach to Cancer Biology and Therapeutic Intervention

1999-present: Research Scientist III, Group Leader, Agensys Inc.
Reserch Interests: Functional Validation of Novel and Recently Discovered Genes as Therapeutic Targets for Treatment of Cancer

PUBLICATIONS:

1. Faris M. and B.S. Zwilling. Somatic Cell Hybrids between Macrophages from Bcg^r and Bcg^s Mice: Characterization of MHC Class II Expression. Cellular Immunology, 1990, 127:120.

2. Faris M. and B.S. Zwilling. Characterization of the Persistent 1-A Expression by Macrophages from Bcg^r Mice. J. Leuk. Biol., 1990, 49:289.

3. Zwilling B.S., M. Dinkins, R. Christner, M. Faris, A. Griffin, M. Hillberger, M. McPeek and D. Pearl. Restraint Stress Induced Suppression of MHC Class II Expression by Murine Peritoneal Macrophages. J. Neuroimmunology, 1990, 29:125.

4. Zwilling B.S., D. Brown, R. Christner, M. Faris, M. Hillberger, C. Van Epps and B.A. Hartaub. Differential Effect of Restraint Stress on MHC Class II Expression by Murine Peritoneal Macrophages. Brain Behavior and Immunity, 1990, 4:330.

5. Faris M. and B.S. Zwilling. Characterization of the Induction of Persistent MHC class II Expression by Hybrids of Macrophages from Bcg^r and Bcg^s Mice. Euro. J. Immunol., 1991, 21:1047.

6. Faris M., F. Gaskin, R.S. Geha and S.M. Fu. Tyrosine Phosphorylation Defines a Unique Transduction Pathway in Human B Cells Mediated via CD40. Trans. Assoc. Amer. Phys. 1993, 106:187.

7. Brown D., M. Faris, M. Hilburger and B.S. Zwilling. The Induction of Persistent 1-A Expression by Macrophages from Bcg^r Mice Occurs via a Protein Kinase C Dependent Pathway. J. Immunol., 1994, 152:1323.

8. Faris M., F. Gaskin, J.T. Parsons and S.M. Fu. CD40 Signaling Pathway: Anti-CD40 mAb Induces Rapid Dephosphorylation and Phosphorylation of Tyrosine-Phosphorylated Proteins Including Protein Tyrosine Kinase Lyn, Fyn and Syk and the Appearance of a 28kD Tyrosine Phosphorylated Protein. J. Exp. Med., 1994, 179:1923.

9. Faris, M., B. Ensoli, N. Stahl. G. Yancopoulos, A. Nguyen, S. Wang and A. Nel. Differential Activation of the ERK, JNK and JAK-Stat Pathways by Oncostatin M and Basic Fibroblast Growth Factor in AIDS-Related Kaposi's Sarcoma cells. AIDS, 1996, 10:370.

10. Faris, M., N. Kokot, L. Lee and A.E. Nel. Regulation of IL-2 transcription by inducible

stable expression of dominant negative and dominant active MEKK-1 in Jurkat T cells. Evidence for the importance of Ras in a pathway which is controlled by dual receptor stimulation. J. Biol. Chem., 1996, 271:27366.

11. Faris, M., N. Kokot, N. Stahl and A.E. Nel. Involvement of Stat3 in Interleukin-6-induced IgM production in a human B cell line. Immunol., 1997, 90:350.

12. Faris, M., B. Ensoli, N. Kokot and A.E. Nel. Inflammatory Cytokines Induce AP-1 Response Elements: Activation of the bFGF promoter and expression of various bFGF isoforms in Kaposi sarcoma and endothelial cells. AIDS, 1998, 12:19.

13. Faris, M., N. Kokot, K. Latinis, S. Kasibhatla, D. Green, G. Koretzky and A.E. Nel. The JNK Cascade Plays a Role in Stress-induced Apoptosis in Jurkat Cells by Upregulating FasL Expression. J. Immunol, 1998, 160:134.

14. Faris, M., B. Ensoli, J. Said, N. Kokot and A.E. Nel. Dominant active Ras affects the life-span, growth factor production and induces Kaposi's sarcoma characteristics in endothelial cells. Cancer Res., 1997, submitted.

15. Ng, D., N. Kokot, M. Faris, A. Saxon and A. Nel. Macrophage Activation by Polycyclic Aromatic Hydrocarbons: Evidence for the involvement of stress-activation protein kinases, AP-1 and anti-oxidant response elements. J. Immunol., 1998, 161: 942.

16. Faris, M., K. M. Latinis, S. Kempiak, G. A. Koretzky and Andre Nel. Stress-Induced Fas Ligand Expression in T cells is Mediated Through A MEKK1-Regulated Response Element in the Fas Ligand Promoter. Mol. Cell. Biol., 1998,18: 5414.

17. Shau, H., A.C. Huang, M. Faris, R. Nazarian., J. de Vellis and W. Chen. Thioredoxin peroxidase (natural killer enhancing factor) regulation of activator protein-1 function in endothelial cells. Biochem. Biophys. Res.Commun. 1998, 249: 683.

18. Abreu-Martin, M., A. Palladino, M. Faris, N. Carramanzana, A. Nel, and S.R. Targan. Fas Activates the JNK pathway in Human Colonic Epithelial Cells: Lack of a Direct Role on Apoptosis. Am. J. Physiol., 1999, 276: 599.

ABSTRACTS:

1. M. Faris and B.S. Zwilling. Characterization of MHC Class II Expression by MacrophageHybrids. J. Leuk. Biol. 46: 314(86), 1989.

2. M. Faris and B.S. Zwilling. Regulation of the Induction of Persistent Ia Expression by Macrophages from Micc that are Resistant to Mycobacterium bovis Strain (BCG). FASEB, 4:1752, 1990.

3. M. Faris and B.S. Zwilling. Continuous Expression of MHC class II Glycoproteins by Macrophage-Hybrids: Regulation of the Induction of the Bcg Gene. Proc. Biomed. Res. Society, 1990.

4. B.S. Zwilling and M. Faris. Characterization of the Induction of Persistent I-A Expression by Macrophages from Bcg^r Mice. J. Leuk. Biol., 48:52, 1990.

5. M. Faris and B.S. Zwilling. The Induction of Persistent Expression of MHC Class II (I-A) Glycoproteins is Mediated by Protein Kinase C (PK-C). FASEB, 5:5614, 1991.

6. M. Faris. The Induction of Persistent MHC class II Expression by rIFN-γ is Dependent on a Protein Kinase C Mediated Pathway. The Graduate Research Forum (OSU), 1991.

7. M. Faris, F. Gaskin, R. S. Geha and S. M. Fu. Phosphorylation of a 28kD Protein by a CD40 Mediated Tyrosine Kinase Pathway. J. Immunol., 150:556, 1993.

8. M. Faris, F. Gaskin, R. S. Geha and S. M. Fu. Tryosine Phosphorylation Defines a Unique Transduction Pathway in Human B Cells Mediated via CD40. Clinical Research, 41:277A, 1993.

9. M. Faris and S.M. Fu. CD40 Signal Transduction: Association of CD40 with Lyn, PI3K, GAP and PLCγ. Clinical Research, 42:206A, 1994.

10. M. Faris, S. Wang and A. Nel. The Oncostatin M Induced Proliferative Response in Kaposi's Sarcoma Cells Involves Adaptor Proteins, Raf-1 and MEK-1. Molecular Pathogenesis and Immunology of HIV-1, 1994.

11. M. Faris, S. Wang, A. Nguyen and A. Nel. The Oncostatin M Response in Kaposi's Sarcoma Cells Involves JAKs, Adaptor Proteins, Raf-1 and MEK-1. FASEB, 9:202A, 1995.

12. A. Nel, M. Faris, F. Xu and N. Kokot. IL-4 and IL-6 Utilize Distinct JAK/Stat Pathways to Drive B-cell Differentiation as Determined at the Level of Ig Genes. Cell Growth Symposium, 1996.

13. M. Faris, N. Kokot, L. Lee and A.E. Nel. Regulation of IL-2 Transcription by the JNK Pathway in Jurkat Cells. J. All. Clin. Immunol., 99: LB53, 1997.

14. M. Faris, N. Kokot, K. Latinis, G. A. Koretzky and A.E. Nel. Role of the JNK cascade in stress-induced apoptosis of Jurkat T cells. FASEB J., 12:930A, 1998.

15. A.E. Nel, A. Saxon, D. Ng and M. Faris. Macrophage activation by polycyclic aromatic hydrocarbons: evidence for the involvement of stress-activated protein kinases, AP-1 and anti-oxidant response elements. FASEB J., 12:1062A, 1998.

16. M. Faris, B. Goka and S. Stuart. Gene expression in breast cancer. Clin. Chem. 45: 10, 1999.

17. A. Raitano, I. Vivanco, R. Hubert, E. Chen, M. Faris, D. Saffran, D. Afar and A. Jakobovits. Auto-catalytic cleavage of the androgen regulated TMPRSS2 protease results in its secretion by prostate and colon cancer epithelia. Proc. Amer. Assoc. Cancer Res. 42: 657, 2001

18. M. Faris, P. Velasquez, R. Hubert, D. Saffran, A. Raitano and A. Jakobovits. Validation of STEAP-1 as a therapeutic target.

19. M. Faris, P. Velasquez, P Nolan, R. Hubert, A. Raitano and A. Jakobovits. Validation of STEAP-1 as a Cell Surface Cancer Therapeutic Target. Proc. Amer. Assoc. Cancer Res. 43: 947, 2002.

PAPERS PRESENTED AT NATIONAL MEETINGS:

26th Annual Meeting, Society of Leukocyte Biology, October 15-18, 1989, Marco Island, FL, by M. Faris and B.S. Zwilling.

American Society for Biochemistry and Molecular Biology, The American Association of Immunologists Joint Meeting (FASEB), June 4-7, 1990, New Orleans, LA, by M. Faris and B.S. Zwilling.

27th Annual Meeting, Society of Leukocyte Biology, Twelfth International RES Congress, October 14-18, 1990, Heraklion, Crete, Geece by B.S. Zwilling and M. Faris.

Immunology of Mycobacterial Infections, National Jewish Center of Immunology and Respiratory Medicine, October 1990, Denver, Colorado, by B.S. Zwilling and M. Faris.

Federation of American Societies for Experimental Biology, April 21-25, 1991, Atlanta, GA, by M. Faris and B.S Zwilling.

AAP/ASCI/AFCR Clinical Research Meeting, April 30-May 3, 1993, Washington DC by M. Faris, F. Gaskin, R.S. Geha and S.M. Fu.

American Association of Immunologists, The Clinical Immunology Society Joint Meeting (FASEB), May 21-25, 1993, Denver, Colorado, by M. Faris, F. Gaskin, R.S. Geha and S.M. Fu.

AAP/ASCI/AFCR Clinical Research Meeting, April 29-May 2, 1994, Baltimore, MD by M. Faris and S.M. Fu.

Federation of American Societies for Experimental Biology, April 9-13, 1995, Atlanta, GA by M. Faris, S. Wang, A. Nguyen and A. Nel.

UK-RSA Symposium on Cell Growth Control, January 28-February 1, 1996, Cape Town, RSA by A. Nel, M. Faris, F. Xu and N. Kokot.

AAAI/AAI/CIS Joint Meeting, February 21-26, 1997, San Francisco, by M. Faris, N. Kokot, L. Lee and A.E. Nel.

Federation of American Societies for Experimental Biology, April 18-22, 1998, San Fransisco, by M.Faris, N. Kokot, K. Latinis, G. A. Koretzky and A.E. Nel

PAPERS PRESENTED AT LOCAL MEETINGS:

16th Annual ICSABER Graduate Research Forum, May 8, 1990, OSU, Columbus, OH, by M. Faris and B.S. Zwilling.

5th Annual Graduate Research Forum, April 20, 1991, Fawcett Center, Columbus, OH., by M. Faris.

12th Annual Research Day, Department of Medicine, April 26, 1993, OMNI Hotel, Charlottesville, VA, by M. Faris.

13th Annual Research Day in Internal Medicine, April 25, 1994, OMNI Hotel, Charlottesville, VA, by M. Faris.

Annual UCLA AIDS Institute Symposium: Molecular Pathogenesis and Immunobiology of HIV-1, November 11, 1994, Loews Santa Monica, CA by M. Faris.

SEMINARS:

Department of Microbiology, The Ohio State University, May 30, 1991. Induction of persistent MHC class II expression by macrophages.

Department of Rheumatology, University of Virginia, February 9, 1994. Update in CD40 mediated signaling: Involvement of PTK, PTP and PI3K.

Jonsson Cancer Center, UCLA, February 22, 1996. Involvement of the Stat pathway in B cell differentiation.

Department of Rheumatology, UCLA School of Medicine, October 30, 1996. Role of the JNK cascade in the regulation of IL-2 production in T lymphocytes.

Jonsson Cancer Center, UCLA, December 12, 1996. Regulation of IL-2 expression in Jurkat cells by MEKK1.

Jonsson Cancer Center, UCLA, October 28, 1997. Rolc of the JNK cascade in the apoptosis of T cells.

EuroCancer 1998, Paris, June 4, 1998. Integrated Approach to the Discovery of Cancer Therapeutics.

American Association of Clinical Chemists-Baychem 99, San Francisco, September 24, 1999. Expression Analysis of Cancer Genes.

PATENT APPLICATIONS:

1. Detection of Altered Expression of Genes Regulating Cell Proliferation. 11-1998.
2. Detection of EGF Regulated Genes in Breast Carcinomas. 9-1999.
3. Differential Gene Expression in Prostate Cancer. 2-2000
4. Prostate Cancer Markers. 2-2000.
5. GPCR Up-regulated in Prostate Cancer. 10-2000
6. 36P6D5: Secreted Tumor Antigen. 10-2000
7. 103P2D6: Tissue Specific Protein Highly Expressed in Various Cancers. 2-2000
8. Diagnosis and Therapy Using SGP28-Related Molecules. 10-2000
9. Novel Serpentine Transmembrane Antigens Expressed in Human Cancer. 12-1999
10. 83P5G4: a Tissue Specific Protein Highly Expressed in Prostate Cancer. 2-2000
11. 34P3D7: a Tissue. Specific Protein Highly Expressed in Prostate Cancer. 2-2000

HONORS AND AWARDS:

Honors Tuition Scholarship 1985-1986
ICSABER Graduate Forum Award, 1990.
AFCR Trainee Investigation Award, 1993.

GRANTS AND FUNDING

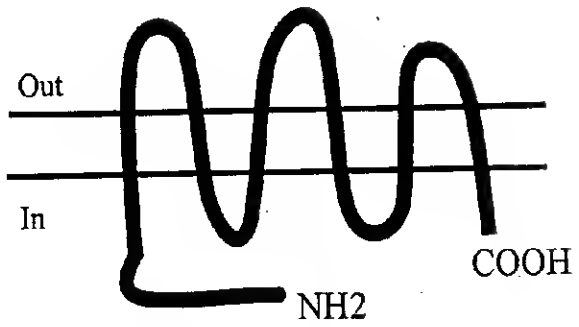
1. NIH-Tumor Immunology Training Grant, 1994. Title: Regulation of Signaling Pathways in Kaposi's Sarcoma. $27,000.
2. NIH-Tumor Immunology Training Grant, 1995. Title: Regulation of Signaling Pathways in Kaposi's Sarcoma. $29,000.
3. NIH Program Project Grant R and D 19, 1998. Title: Role of the JNK Pathway in SLE. $60,000

PROFESSIONAL AFFILIATION:

Society for Leukocyte Biology.
American Federation for Clinical Research.
American Association for the Advancement of Science
American Association of Immunologists
Jonsson Cancer Center

STEAP-2 Characteristics

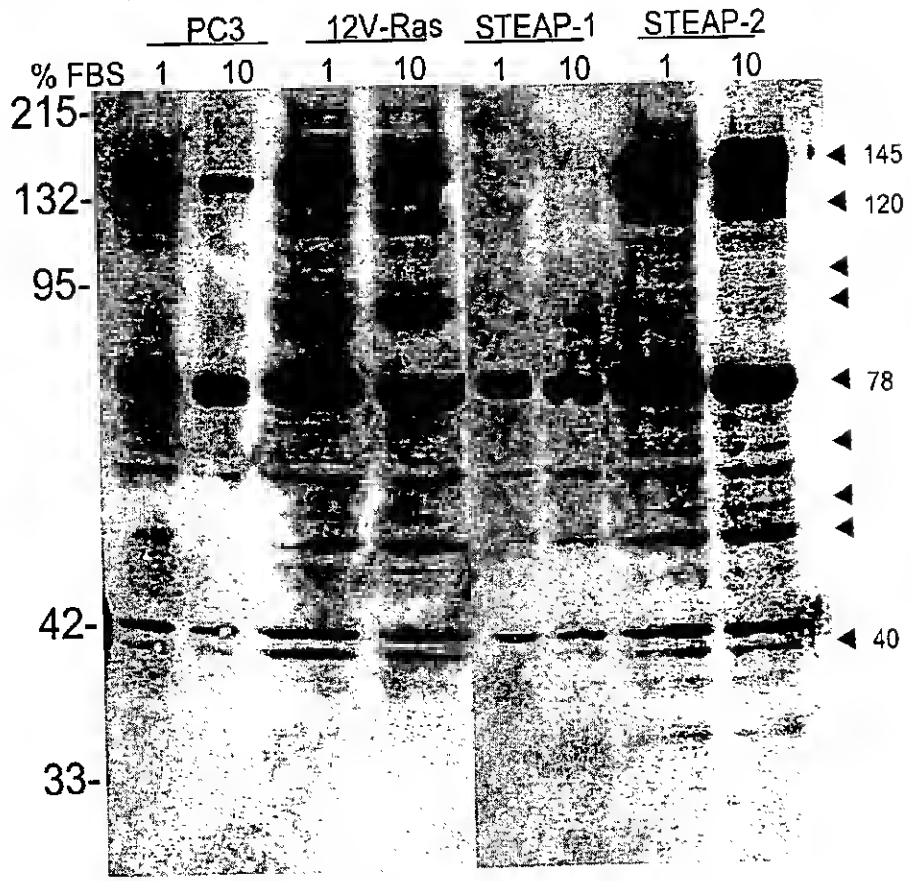
- The expression of STEAP-2 in normal tissues is predominantly restricted to the prostate.
- STEAP-2 is expressed in several cancerous tissues:
  - In patient-derived prostate, colon and lung cancer specimens
  - Multiple cancer cell lines, including prostate, colon, Ewing's sarcoma, lung, kidney, pancreas and testis
- By ISH, STEAP-2 expression appears to be primarily limited to ductal epithelial cells.



Exh. 6.+ B -1

Agensys

STEAP-2 Induces Tyrosine Phosphorylation in PC3 Cells

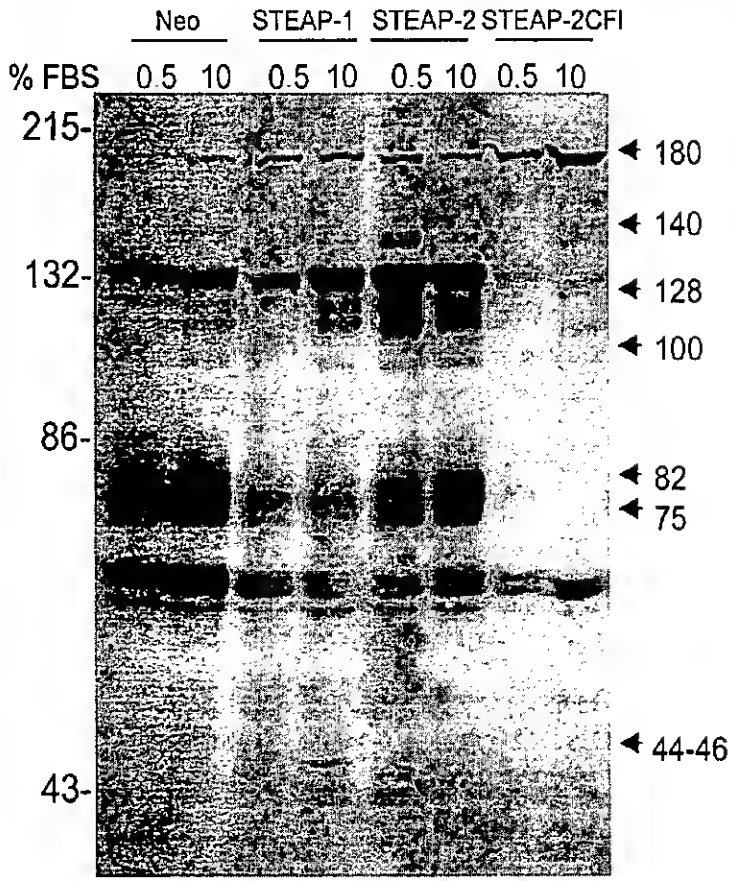


- **STEAP-2 induces the tyrosine phosphorylation of proteins at 140-150, 120, 75-80, 62 and 40 kDa.**

Exhibit B - 2

Agensys

STEAP-2 Enhances Tyrosine Phosphorylation in NIH 3T3 Cells



Anti-Phospho PY Blot

- STEAP-2 enhances the phosphorylation of p135-140, p78-75 by STEAP-2 in NIH 3T3 cells.
- STEAP-2 C-Flag enhances the phosphorylation of p180, and induces the de-phosphorylation of p132, p82 and p75.

Exhibit B - 3

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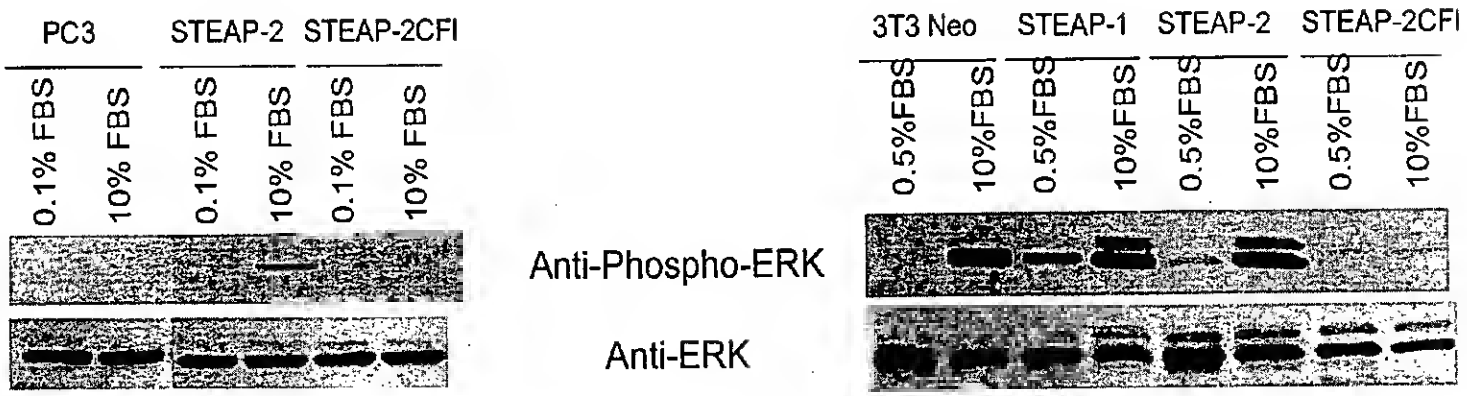
STEAP-2 Induces ERK Phosphorylation



- STEAP-2 Induces ERK phosphorylation in PC3 and 3T3 cells in 0.5 and 10% FBS.
- Lack or ERK phosphorylation in 3T3-STEAP-2-cflag cells. Potential role as dominant negative.

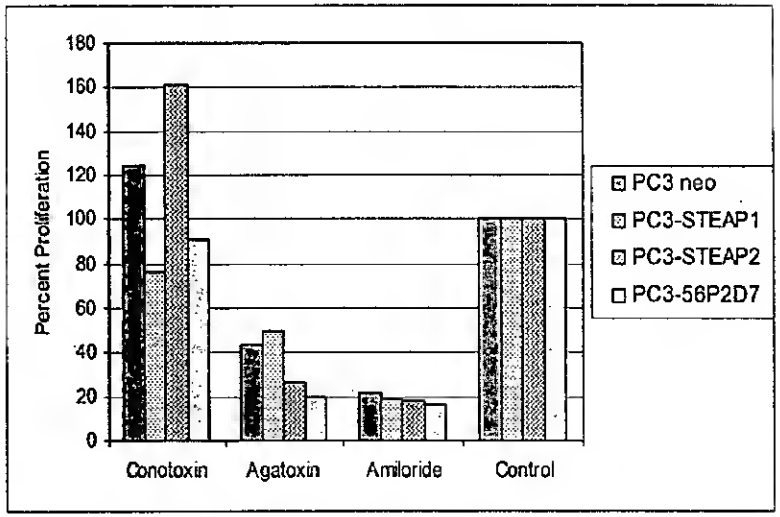
Exhibit B - 4

STEAP Enhances Calcium Flux in PC3 cells

Calcium Flux

Cell line	Ca flux After LPA % Responding Cells
PC3-neo	22.9
PC3-STEAP-1	40.1
PC3-STEAP-2	47.9
PC3-CAT	46.9

Percent Proliferation



- PC-STEAP-1 and PC3-STEAP-2 exhibit enhanced calcium flux in response to LPA.
- PC3-STEAP-1 demonstrates susceptibility to the L type calcium channel inhibitor, conotoxin.
- PC3-STEAP-2 shown susceptibility to the PQ type calcium channel inhibitor, agatoxin.
- NDGA and TEA had no effect on the proliferation of PC3-STEAP-2 cells.

Exhibit B - 5

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STEAP-2 Alters the Effect of Paclitaxel on PC3 Cells

	Percent Survival		
	Neo	STEAP-1	STEAP-2
Doxonbicin 5uM	45.2	28.2	79.2
Doxonbicin 1uM	82.4	90.3	99.9
Doxonbicin 0.5uM	100.3	120.7	117.1
Doxonbicin 0.1uM	111.9	144.2	117.2
Paclitaxel 5uM	5.2	14.1	**44.8**
Paclitaxel 1uM	5.2	13.9	**44.8**
Paclitaxel 0.5uM	5.3	15.7	**47.2**
Paclitaxel 0.1uM	26.3	50.5	**89.8**

Other Chemotherapeutics Tested without yielding a differential response between STEAP-expressing and control cells:

- Flutamide
- Genistein
- Rapamycin

- STEAP-2 confers partial resistance to Paclitaxel in PC3 cells
- Over 8 fold increase in percent survival of PC3-STEAP-2 relative to PC3-Neo cells.

Exhibit B – 6

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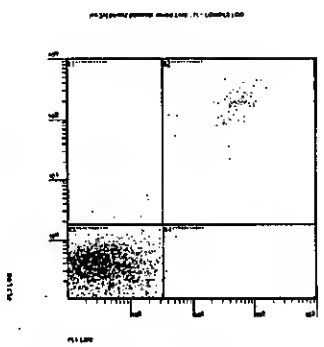
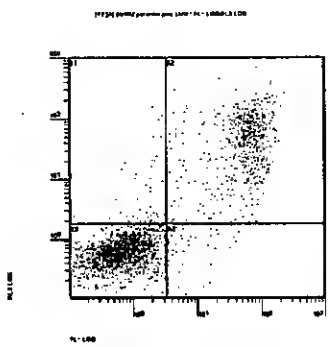
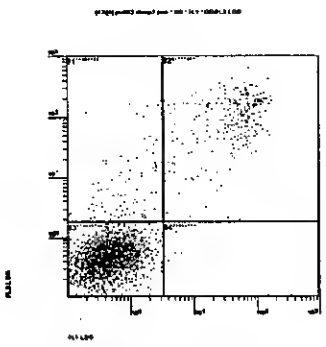
Inhibition of Apoptosis by STEAP-2



PC3-Neo



PC3-Neo+Pac



PC3-STEAP-2+Pac

Cells	% Inhibition of Apoptosis
PC3-neo	0
PC3-STEAP-1	17.6
PC3-STEAP-2	**45.3**
PC3-83P3H3 pCaT	13.2

- PC3 cells were treated with paclitaxel for 60 hours and analyzed for apoptosis by annexinV-PI staining.
- Expression of STEAP-2 partially inhibits apoptosis by paclitaxel.

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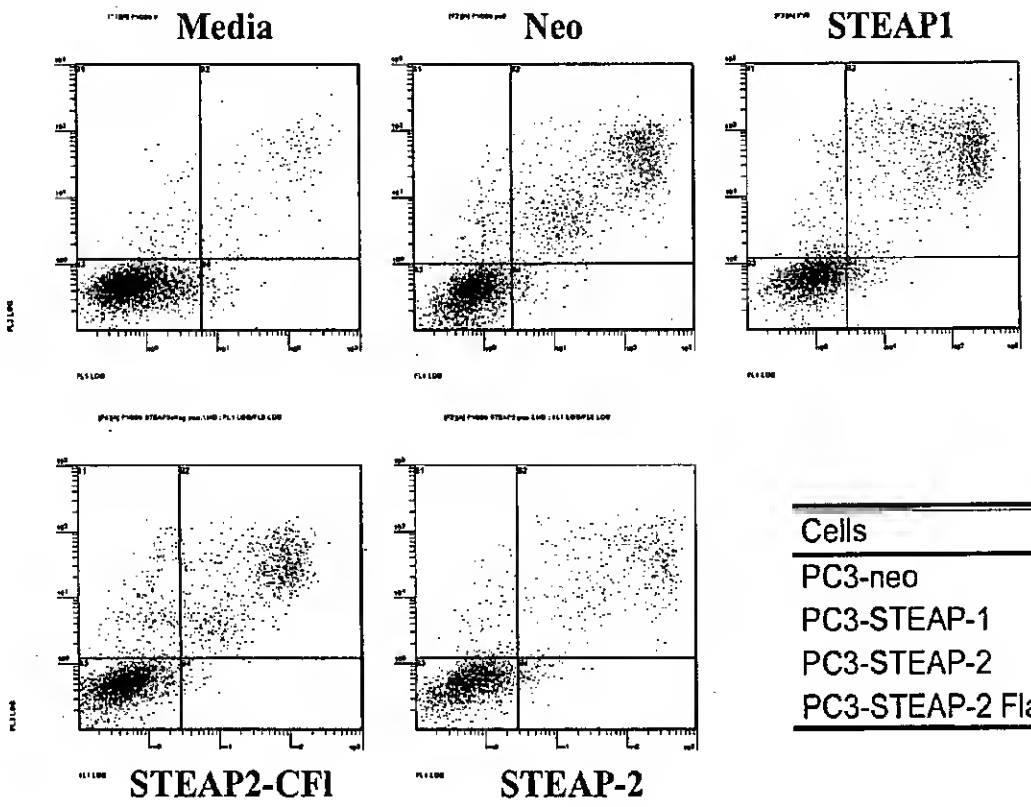
Exhibit B - 7

STEAP-2 Attenuates Paclitaxel Mediated Apoptosis



Cells	% Inhibition of Apoptosis
PC3-neo	0.0
PC3-STEAP-1	5.0
PC3-STEAP-2	47.3
PC3-STEAP-2 Flag	28.4

- PC3 cells were treated with paclitaxel for 68 hours and analyzed for apoptosis.
- Expression of STEAP-2, but not STEAP-2CFlag, partially inhibits apoptosis by paclitaxel.

Exhibit B - 8

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